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Spatial patterns of tissue stable isotope contents give insight into the nutritional sources for seep communities on the Gulf of Mexico lower slope

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ABSTRACT: In this study, we present the first thorough trophic characterization of cold seep macrofaunal communities on the Gulf of Mexico lower continental slope (>1000 m depth). We analyzed tissue $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of vestimentiferan tubeworms, bathymodiolin mussels, vesicomyid clams, and their associated macrofaunal communities from discrete collections made across the entire lower slope. Over half of macrofauna associated with mussels and about half associated with vestimentiferans had $\delta^{13}C$ values below −45‰. We also observed high spatial variability in the $\delta^{13}C$ values of entire local communities, and the $\delta^{13}C$ of associated fauna were significantly correlated with the $\delta^{13}C$ compositions of the symbiotic species from the same location. These data indicate widespread incorporation of methane-derived carbon in mussel and vestimentiferan communities. This finding was particularly surprising in communities associated with older vestimentiferans, given the low rates of seepage observed in similar communities on the upper slope. On average, $\delta^{15}N$ values in mussels and their associates were significantly more depleted and more variable than vestimentiferans, clams, and their associates, and there was a significant linear relationship between tissue $\delta^{15}N$ values of mussels and their associated communities. The tissue $\delta^{34}S$ values in macrofauna associated with vestimentiferans were more variable and significantly more depleted than mussel associates ($\delta^{34}S$ = −16.8 to +19.1‰ for vestimentiferan associates and $\delta^{34}S$ = −3.1 to +20.8‰ for mussel associates), consistent with higher isotopic fractionation during sulfate reduction in vestimentiferan habitats and a potentially higher nutritional contribution of sulfide-derived organic sulfur in vestimentiferan communities.

KEY WORDS: Hydrocarbon seep · Biogenic methane · Sulfide · Vestimentiferan · Bathymodiolin · Vesicomyid · Chemoautotrophy · Methanotrophy

INTRODUCTION

Lush animal communities thrive in areas of natural oil and gas seepage on the Gulf of Mexico continental slope. The expansion of oil and natural gas exploration and extraction into ever-deeper waters of the Gulf increases the potential threat to the communities occupying deeper seep habitats. Seeps occurring at over 1000 m depth on the lower continental slope have historically been less well-studied than shallower seeps, and it is, therefore, necessary to address first-order questions regarding animal distributions and community ecology in order to inform the future conservation of deepwater seep communities. Two research cruises were conducted in 2006 and 2007 as a multidisciplinary effort to discover and explore new sites and to collect data on seep communities across a large geographic and bathymetric range on the Gulf

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of Mexico lower continental slope (700 km east to west and from 970 to 2800 m depth).

As part of this effort, we used stable isotope analysis of animal tissues to elucidate the nutritional sources in macrofaunal communities associated with 3 dominant seep taxa: vestimentiferan tubeworms, bathymodiolin mussels, and vesicomyid clams. These taxa contain bacterial endosymbionts that harness energy from reduced chemicals in seeping fluid to fix carbon and provide nutrition for their hosts. Mussels and vestimentiferans dominate biomass in Gulf of Mexico seep communities and act as foundation species, providing habitat for diverse assemblages of macrofauna (Bergquist et al. 2003, Bergquist et al. 2005, Cordes et al. 2010a).

The 3 bathymodiolin mussel species that occur on the lower slope are *Bathymodiolus childressi*, which is also common at shallower depths (overall depth range 525 to 2284 m), *B. brooksi* (1080 to 2745 m depth), and *B. heckerae* (2180 to 2745 m depth) (Cordes et al. 2010a). Where the depth ranges of these species overlap, *B. brooksi* often co-occurs in the same aggregations with either *B. childressi* or *B. heckerae*, but the latter 2 species have never been found to co-occur. *B. childressi* has only methanotrophic symbionts, which use methane as both an energy and carbon source for carbon fixation (Cavanaugh et al. 1987); *B. brooksi* forms a dual symbiosis with methanotrophic bacteria and sulfur-oxidizing chemoautotrophic bacteria, which use reduced sulfur species as an energy source and dissolved carbon dioxide as a carbon source (Fisher et al. 1993), and *B. heckerae* contains 4 different symbionts: a methanotroph, 2 chemoautotrophs, and one methylotroph-related phylotype (Duperron et al. 2007). Mussel beds can be many layers thick, and because mussels lack binding proteins to transport molecules to their symbionts, they require sufficient concentrations of seep fluid and oxygen in the epibenthic water to support autotrophy. A community of macrofauna (animals ≥1 mm in size) inhabits the mussel shells and interstices between them and is therefore also exposed to these seeping chemicals.

There are 3 vestimentiferan species on the lower slope: *Escarpia laminata* and *Lamellibrachia* sp. 1 are common and frequently co-occur in the same aggregations, and *Lamellibrachia* sp. 2 is rare and occurs with the other two (Miglietta et al. 2010). All vestimentiferans contain sulfide-oxidizing chemoautotrophic endosymbionts that provide the bulk of the vestimentiferans' nutrition. The associated macrofaunal community inhabits the chitinous tubes and interstices of vestimentiferan aggregations. Since older vestimentiferans can grow up to a meter above the sediment surface and typically inhabit less active seep habitats than mussels, the associated fauna generally experience less exposure to seeping fluid than mussel associates.

Vesicomyid clams are less common than mussels and vestimentiferans and are typically found burrowing through the sediment leaving distinctive trails. It has been hypothesized that since they acquire sulfide through their foot, the clams must move around because there is insufficient flux of seeping fluids to replenish the sulfide in one location (Fisher 1990). Animals associated with vesicomyid clams in the Gulf of Mexico either inhabit the sediment surrounding the clams or colonize the exposed portion of the clam shells.

Opportunistic quantitative collections of vestimentiferan and mussel communities were analyzed to examine community composition and diversity (Cordes et al. 2010a), and subsamples of animal tissues from 11 vestimentiferan and 20 mussel aggregations were analyzed for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ content to examine the nutritional sources of the associated community. Additionally, we analyzed tissue stable isotopes of vesicomyid clams and their associates collected from 4 locations.

Becker et al. (2010, 2011) found unexpectedly high spatial variability in the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ contents of vestimentiferans and mussels across the lower slope, reflecting variability in the inorganic carbon, nitrogen, and sulfur sources that support them. These studies also demonstrated widespread incorporation of biogenic methane, even in vestimentiferans, whose sulfide-oxidizing symbionts cannot directly fix methane, but likely use porewater dissolved inorganic carbon derived from free-living microbial methane oxidation (Dattagupta et al. 2006, Becker et al. 2011).

Previous studies on the upper slope tie nutrition of seep-associated macrofauna, both mobile and sessile, to seep primary production, though some incorporation of surface-derived nutrition occurs (Levin & Michener 2002, Levin 2005, MacAvoy et al. 2005, Levin & Mendoza 2007, Carlier et al. 2010, Cordes et al. 2010b). Limited direct trophic transfer occurs from the dominant symbiotic species to macrofaunal consumers, and associated communities are supported instead by free-living chemoautotrophic and methanotrophic bacteria, which exhibit taxonomic and functional diversity and variable stable isotope values (House et al. 2009, Orcutt et al. 2010). The macrofaunal community occupies a variety of trophic niches, some of which may integrate nutritional sources across seep and non-seep habitats.

One goal of this study was to assess whether the local macrofaunal communities associated with the symbiotic taxa reflect similar spatial variability and apparent use of biogenic methane-derived carbon observed in vestimentiferans and mussels. Given the functional autotrophy in vestimentiferans and mussels, they take up inorganic molecules directly from the environment for their symbionts to fix into organic molecules. Thus, multiple factors could decouple isotope values in vestimentiferans and mussels from the isotope values of free-living bacteria and hence the macrofaunal consumer community. For instance, vestimentiferans can use their long 'roots' to mine for inorganic molecules deep in the sediment that would not be available to microbes at the sediment-seawater interface, or mussels and vestimentiferans could select for specific inorganic compounds, such as nitrate or ammonium, that differ from sources used by free-living microbes. Understanding these patterns in spatial variability will help to discern the nutritional sources for both symbiotic and non-symbiotic seep animals and provide insight into the underlying geochemical processes that operate at different spatial scales to drive these patterns.

MATERIALS AND METHODS

Study sites

The study sites are named according to the Bureau of Ocean Energy Management lease block designations. Each name includes a 2-letter abbreviation for the region (e.g. GC for Green Canyon) followed by a 3-digit number. The 13 sites in this study are located along the lower continental slope of the Gulf of Mexico from 225 km south of Texas, USA, near the Texas–Louisiana border to south of Alabama (Fig. 1). Sites ranged in depth from 970 m to 2800 m. Descriptions of the study sites are given in Becker et al. (2010) and Roberts et al. (2010).

Community collections

Collections were made in 2006 using the deep submergence vehicle 'Alvin' and in 2007 using the remotely operated vehicle (ROV) 'Jason II.' Quantitative collections of vestimentiferan and mussel communities were obtained using the Bushmaster Jr. and mussel pot/mussel scoop collection devices, respec-

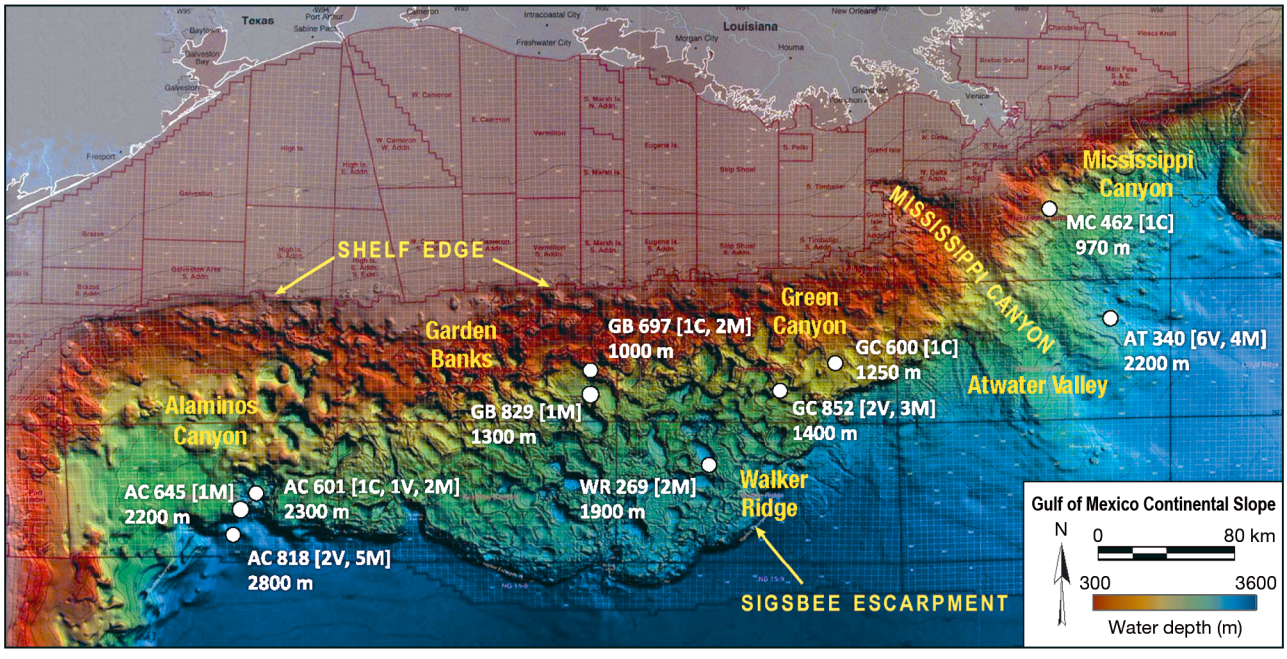

Fig. 1. Collection sites on the Gulf of Mexico lower continental slope. Site names are based on Bureau of Ocean Energy Management (BOEM) lease block designations and consist of a 2-letter abbreviation, which stands for the region (e.g. AC = Alaminos Canyon), followed by a 3-digit number. Yellow text gives the name for the region; white points and text signify the specific study sites. Notations inside the brackets indicate how many of each community type was collected at a site: C = vesicomyid clams (*Calyptogena ponderosa* and an undescribed vesicomyid), M = mussels (*Bathymodiolus* spp.), and V = vestimenitiferans (*Escarpia laminata* and *Lamellibrachia* spp.). Bathymetric depth is given in meters below the site name

tively (Cordes et al. 2010a). The Bushmaster Jr. is a hydraulically actuated collection device with an open diameter of 0.7 m and lined with a 63-µm mesh (Bergquist et al. 2003, Urcuyo et al. 2003). The device is placed over a clump of vestimentiferans and then closed to capture their and all animals associated with their tubes and interstices. The mussel pot device (Cordes et al. 2010a), as modified from the design of Van Dover (2002), was used to collect mussels. This cylindrical aluminum pot is 25 cm in diameter, 30.5 cm in height, and has an internal volume of 0.015 m^3. The submersible or ROV's manipulator places the device over a clump of mussels and turns a handle one full rotation to close a Vectran™ skirt. Because some of the mussel communities contained very large mussels that were not effectively captured with the mussel pot, a 'mussel scoop' was used to collect some of the community samples (Cordes et al. 2010a). The mussel scoop is a coarse mesh net with an opening of approximately 700 cm^2 lined with a pillowcase. The mussel communities collected with the 2 different sampling devices were not significantly different in composition (Cordes et al. 2010a). The manipulator of the submersible dragged the scoop through the mussel bed, then placed the entire scoop into an insulated biobox and closed the lid. Vesicomyid clams and their associated communities were sampled using the mussel scoop (AC601 collection) or directly with the submersible's manipulator (other 3 collections). Only associated fauna attached to the clam shells were obtained from manipulator collections.

Once onboard the ship, Bushmaster collections were processed for community composition as in Cordes et al. (2010a). Associated fauna were rinsed from tubeworm tubes, passed through a 1 mm sieve, sorted, and identified to the lowest possible taxonomic level, which most often was genus. Up to 3 individuals of each taxon from each collection were sampled for stable isotope analysis. Stable isotope samples were obtained from associated fauna by dissecting a piece of muscle tissue from large animals or using whole individuals for smaller animals. The samples were rinsed with deionized water to remove any residual seawater and frozen at −70°C. For the vestimentiferans, vestimentum (muscle) tissue was sampled from up to 6 individuals of each species from each collection.

Mussel pot and mussel and clam scoop collections were handled similarly. All mussels and clams in these collections were opened to check for commensal polynoids (*Branchipolynoe seepensis*) and nautilliniellid polychaetes, and mantle tissue was sampled from up to 6 individuals of each species from each collection.

Stable isotope analysis

All samples were dried at 60°C, homogenized, and acidified to remove inorganic carbonate. Samples were redried and subsamples were analyzed for stable carbon and nitrogen isotopes at the Stable Isotope Facility at the University of California, Davis, using an Integra elemental analyzer coupled with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon) or by R. W. Lee (School of Biological Sciences, Washington State University) using continuous-flow isotope ratio mass spectrometry with a Costech elemental analyzer coupled to a Micromass Isoprime isotope ratio mass spectrometer (EA/IRMS). Data from each of the laboratories are calibrated to NIST (National Institute of Standards and Technology) reference materials. All stable sulfur isotope analyses were performed by S. A. Macko at the University of Virginia Stable Isotope Laboratory using continuous-flow isotope ratio mass spectrometry with a Carlo Erba elemental analyzer coupled to a Micromass Optima EA/IRMS.

Values are expressed using δ (delta) notation and reported in units of permil (‰), where:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 10^3 \quad (1)$$

where $X = {}^{13}C$, ^{15}N, or ^{34}S and $R = {}^{13}C/{}^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$. PDB (Pee Dee Belemnite) was used as the standard for carbon, air N_2 for nitrogen, and CDT (Canyon Diablo triolite) for sulfur.

Statistical analysis

Pairwise comparisons of isotope values of all associated macrofauna were made between the 3 different symbiotic fauna (vestimentiferans, mussels, and clams) using 2-sample *t*-tests where the data met the assumptions for parametric statistical methods and Mann-Whitney *U*-test where these assumptions were not met (Zar 2010). Because multiple comparisons were made, a sequential Bonferroni correction factor was applied to determine the p-value at which differences were considered significant (Holm 1979).

For the purposes of statistical tests and discussion, a 'collection' is defined as all samples contained in one scoop, Bushmaster, or mussel pot sampling event—in other words, all animals that were collected within a relatively small (<2 m^2) area. To test

whether symbiotic fauna type (vestimentiferans or mussels) or collection location had a significant effect on tissue stable isotope values, we applied general linear models (GLMs) to the individual isotope data (not collection averages). For the first iteration, we separated the data set into 2 parts: (1) symbiotic fauna (i.e. vestimentiferans and mussels) and (2) associated fauna. The response variable was one of the 3 isotope values—$\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$—and the predictor variables were symbiotic fauna and collection (nested within symbiotic fauna). For the second iteration, the data set was split further so that a separate GLM was run for each of the following: vestimentiferans, vestimentiferan associates, mussels, and mussel associates. The only predictor in these models was collection. This was to test whether collection was an important predictor variable for each of these animal groups separately.

To test for a linear relationship between the mean isotope values of associated macrofauna within a collection and the mean isotope values of their symbiotic fauna within the same collection, we performed regression analyses. For these tests, each data point consisted of the mean isotope value ($\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$) of all associated fauna in a single collection as the response variable and the mean isotope values of all of the mussels or vestimentiferans in the same collection as the predictor.

To test whether the mean isotope values of associated fauna were significantly different from the symbiotic fauna (vestimentiferans or mussels) in the same collections, we conducted paired *t*-tests on the mean isotope values of associated and symbiotic fauna where assumptions for parametric statistics were met. Where assumptions for parametric statistics were not met, the differences between the mean isotope values of the associated and symbiotic fauna were calculated for each collection, and a Wilcoxon signed rank test was performed to test whether the median of the differences differed significantly from 0.

RESULTS

Overall trends

A map of the collection locations is shown in Fig. 1. The majority of tissue isotope values for seep-associated macrofauna were more depleted than the isotope values for photosynthesis-derived particulate organic matter (POM), which has $\delta^{13}C$ = −22 to −15‰ (Gearing et al. 1984), $\delta^{15}N$ = 2.5 to 23.3‰ at depth >200 m (Saino & Hattori 1987), and $\delta^{34}S$ = 10 to 20‰ (Fry et al. 1983, Peterson & Fry 1987) (Table S1 in the Supplement at www.int-res.com/articles/suppl/m498p133_supp.pdf, Fig. 1). Pairwise comparisons of isotope values in all associated fauna between the different symbiotic fauna types revealed significant differences between all pairs except between $\delta^{34}S$ values in vestimentiferan and clam associates (Table S2 in the Supplement).

$\delta^{13}C$ in mussel and vestimentiferan communities

Tissue $\delta^{13}C$ values of associated fauna showed a similar pattern of spatial variability to the symbiotic fauna with which they were collected. Mean $\delta^{13}C$ values of associated macrofauna were linearly related to the $\delta^{13}C$ values of the mussels ($p < 0.001$, $R^2 = 0.84$) or vestimentiferans ($p < 0.001$, $R^2 = 0.77$) with which they were collected (Table 1, Fig. 2a). GLMs revealed that both symbiotic fauna type (either mussel or vestimentiferan) and collection (nested within symbiotic fauna type) were significant factors in determining tissue $\delta^{13}C$ values of symbiotic and associated fauna (Table S3 in the Supplement). Collection also significantly affected $\delta^{13}C$ when each animal category was considered separately (i.e. vestimentiferans only, mussels only, vestimentiferan associates, and mussel associates) (Table S3).

Table 1. Regression analysis between the mean $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of associated macrofauna (assoc.) and the mean stable isotope values of the symbiotic fauna in the same collection. vest: vestimentiferans

	Factor	Value	*t*-statistic	p-value	R^2
$\delta^{13}C$					
Assoc. vs. vest.	Slope	0.6	5.41	<0.001	0.77
	Intercept	3.7	−2.58	0.030	
Assoc. vs. mussels	Slope	0.9	9.62	<0.001	0.84
	Intercept	−13.9	1.13	0.272	
$\delta^{15}N$					
Assoc. vs. vest.	Slope	0.3	0.65	0.531	0.05
	Intercept	4.8	2.81	0.020	
Assoc. vs. mussels	Slope	0.5	4.20	0.001	0.49
	Intercept	3.1	7.12	<0.001	
$\delta^{34}S$					
Assoc. vs. vest.	Slope	0.6	5.46	<0.001	0.77
	Intercept	−13.9	−2.55	0.031	
Assoc. vs. mussels	Slope	0.9	9.01	<0.001	0.82
	Intercept	3.7	0.64	0.529	

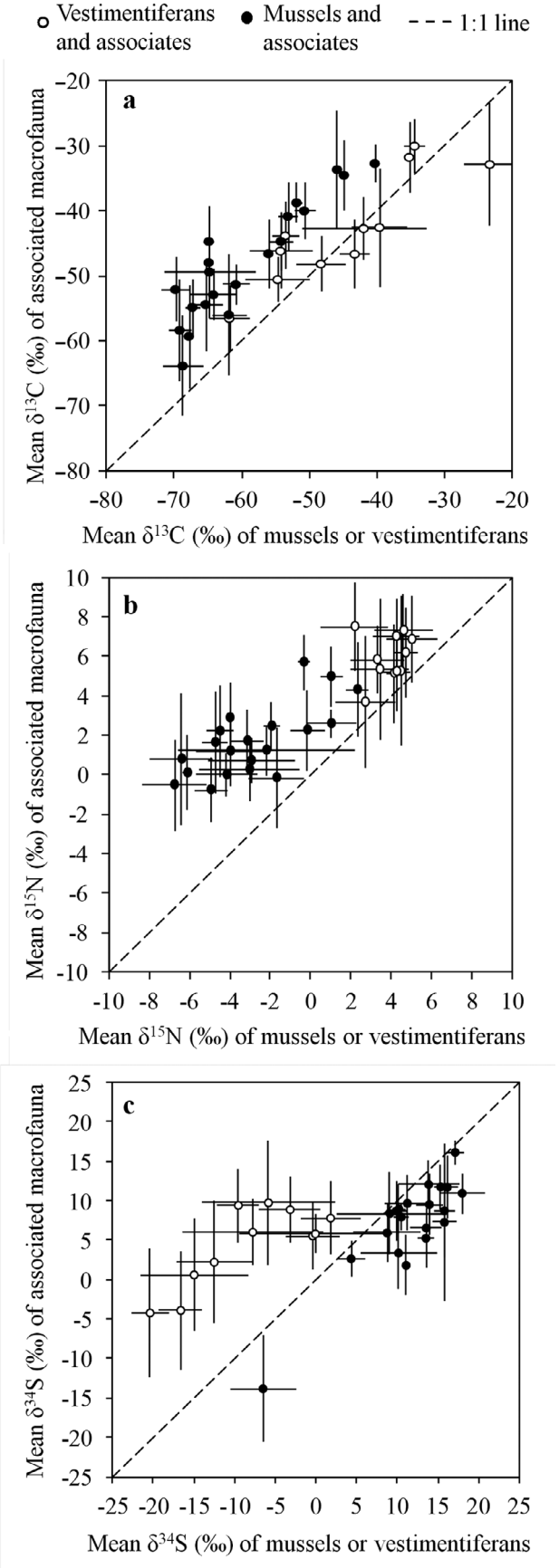

Fig. 2. Mean and standard deviation (SD) of tissue (a) $\delta^{13}C$, (b) $\delta^{15}N$, and (c) $\delta^{34}S$ values of fauna in each vestimentiferan and mussel collection. Each symbol represents the mean tissue stable isotope values for one collection, with the mussels or vestimentiferans on the *x*-axis and the associated macrofauna from the same collection on the *y*-axis. The dotted 1:1 line represents equal values on the *x*- and *y*-axes

Paired *t*-tests between mean associated and symbiotic fauna tissue $\delta^{13}C$ by collection revealed that in vestimentiferan collections, mean associate $\delta^{13}C$ values were not significantly different from the vestimentiferans with which they were collected ($p = 0.165$), but mussel associates had significantly greater $\delta^{13}C$ values than the mussels by an average of 11.2 ± 4.6‰ SD ($p < 0.001$) (Table S4 in the Supplement). Specifically, the data points of the mean associated fauna $\delta^{13}C$ vs. mean vestimentiferan $\delta^{13}C$ do not significantly differ from the 1:1 line, while the points for mussel collections all lie above the 1:1 line (Fig. 2a).

$\delta^{34}S$ in vestimentiferan and mussel communities

Mussel $\delta^{34}S$ values ranged from −10.9 to +21.1‰, and vestimentiferan $\delta^{34}S$ ranged from −23.1 to +18.4‰ (Table 2, Fig. 3d). Excluding the mussel collection from AC645 that had notably more depleted $\delta^{34}S$ values (−10.9 to −2.8‰), the remaining mussels only ranged from $\delta^{34}S = 0.9$ to 21.1‰. Tissue $\delta^{34}S$ of vestimentiferan associates ranged from −16.3 to +19.1‰, and mussel associates ranged from $\delta^{34}S = -18.5$ to +21.1‰ with the AC645 collection included and $\delta^{34}S = -3.1$ to +20.8‰ without it (Fig. 3b). Regression analysis showed a significant linear relationship between mean $\delta^{34}S$ values of vestimentiferans and the mean $\delta^{34}S$ values of their associates ($p < 0.001$, $R^2 = 0.77$, Table 1). This relationship was also significant for the mean $\delta^{34}S$ values of mussels and their associates ($p < 0.001$, $R^2 = 0.82$, Table 1). Omitting the AC645 mussel collection gave a weaker, but still significant, linear relationship ($p = 0.004$, $R^2 = 0.40$). GLMs indicated that symbiotic fauna type and collection were significant factors in determining $\delta^{34}S$ values for vestimentiferans, mussels, and their associates when considered both together and separately (Table S3).

Paired *t*-tests between the means of associated and symbiotic fauna $\delta^{34}S$ by collection revealed significantly greater $\delta^{34}S$ values in the associated macrofauna than the vestimentiferans ($p < 0.001$), whereas $\delta^{34}S$ values were significantly lower in the associated fauna than the mussels with which they were col-

Table 2. Mean, standard deviation, and range of the tissue stable isotope values for the symbiotic fauna species from all collections. N_{CN} is the number of individuals analyzed for carbon and nitrogen isotopes; N_S: number of individuals analyzed for sulfur isotopes

Species	N_{CN}	N_S	$\delta^{13}C$ (‰)				$\delta^{15}N$ (‰)				$\delta^{34}S$ (‰)			
			Mean	±SD	Min.	Max.	Mean	±SD	Min.	Max.	Mean	±SD	Min.	Max.
Bathymodiolus brooksi	57	57	−59.5	±8.5	−72.3	−40.2	−4.2	±2.3	−8.9	2.2	11.4	±6.0	−10.9	21.1
Bathymodiolus childressi	18	18	−62.7	±8.5	−72.2	−45.8	0.2	±1.8	−3.3	2.9	12.6	±5.0	4.9	20.5
Bathymodiolus heckerae	13	8	−61.2	±7.0	−67.6	−44.1	−1.8	±1.5	−4.8	0.6	8.4	±5.3	2.3	16.2
Calyptogena ponderosa	9	9	−35.7	±0.9	−36.7	−34.4	−1.0	±4.4	−1.0	4.4	6.2	±3.1	1.1	12.8
Undescribed vesicomyid	3	3	−34.7	±0.6	−35.4	−34.4	1.2	±1.2	0.0	2.2	−8.2	±5.7	−14.1	−2.9
Escarpia laminata	50	44	−42.0	±13.0	−65.6	−19.5	4.4	±0.8	3.1	6.2	−6.1	±10.3	−23.1	18.4
Lamellibrachia sp. 1	14	14	−43.3	±7.4	−55.2	−32.5	1.9	±1.3	0.1	3.8	−10.0	±8.6	−22.3	5.5
Lamellibrachia sp. 2	1	1	−42.5				3.6				−12.7			

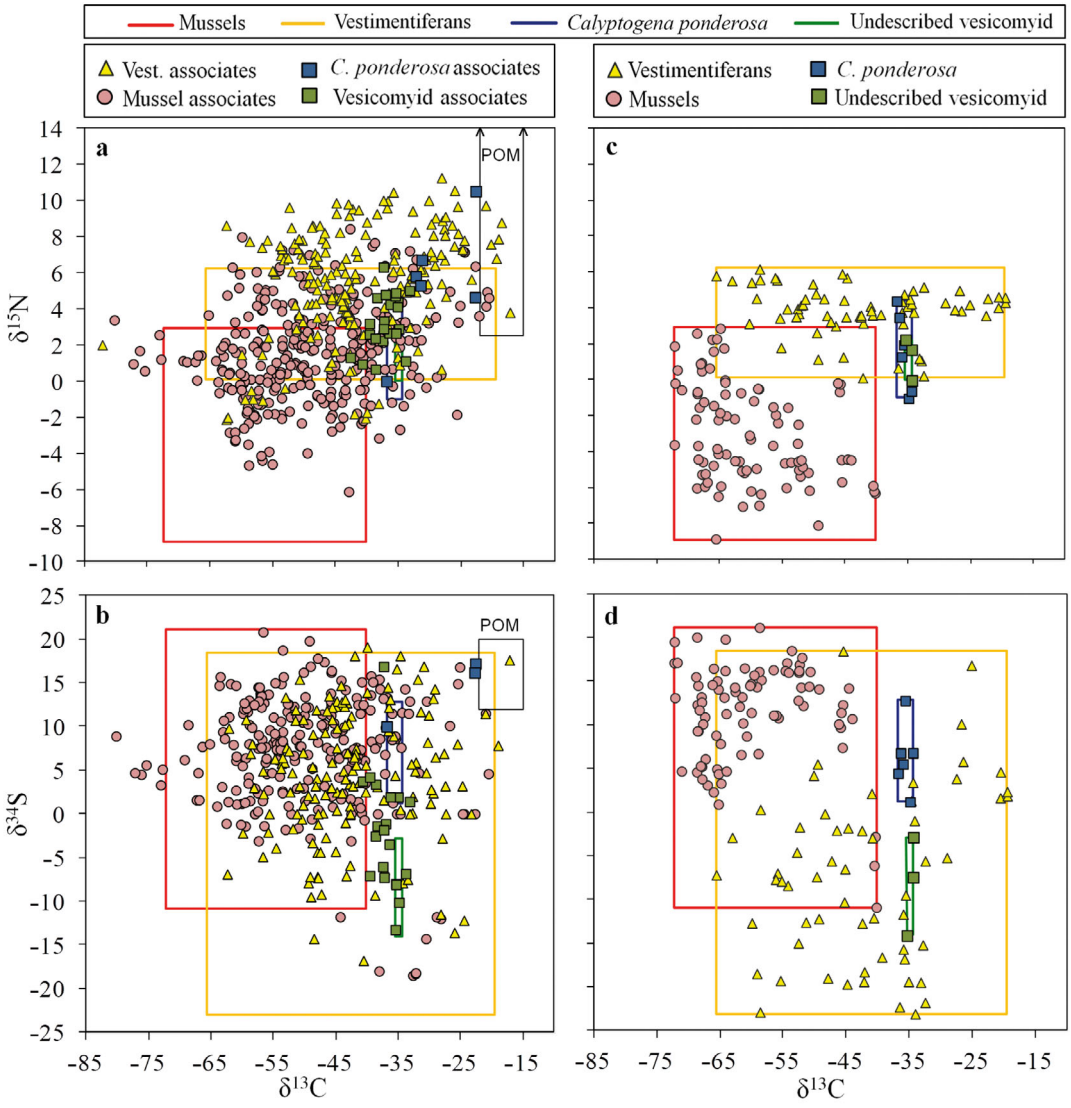

Fig. 3. Tissue (a) $\delta^{15}N$ and (b) $\delta^{34}S$ vs. $\delta^{13}C$ values for all animals sampled in this study. The rectangles represent the range of values for symbiotic fauna and literature values for photosynthesis-derived particulate organic matter (POM). POM $\delta^{13}C$ = −22 to −15‰ (Gearing et al. 1984), $\delta^{15}N$ = 2.5 to 23.3‰ at depth >200 m (Saino & Hattori 1987), and $\delta^{34}S$ = 10 to 20‰ (Fry et al. 1983, Peterson & Fry 1987). The maximum range of $\delta^{15}N$ values for POM exceeds the range shown in panel (a), which is indicated by the arrowheads at the top of the POM rectangle. Points in (a) and (b) represent the associated macrofauna, with each point representing one individual stable isotope sample. The associated fauna comprised 54 taxa from 10 phyla, 36 of which were identified to genus or species level (Table S1 in the Supplement; www.int-res.com/articles/suppl/m498p133_supp.pdf). In (c) and (d), points represent individual vestimentiferan (vest.), mussel, or clam samples

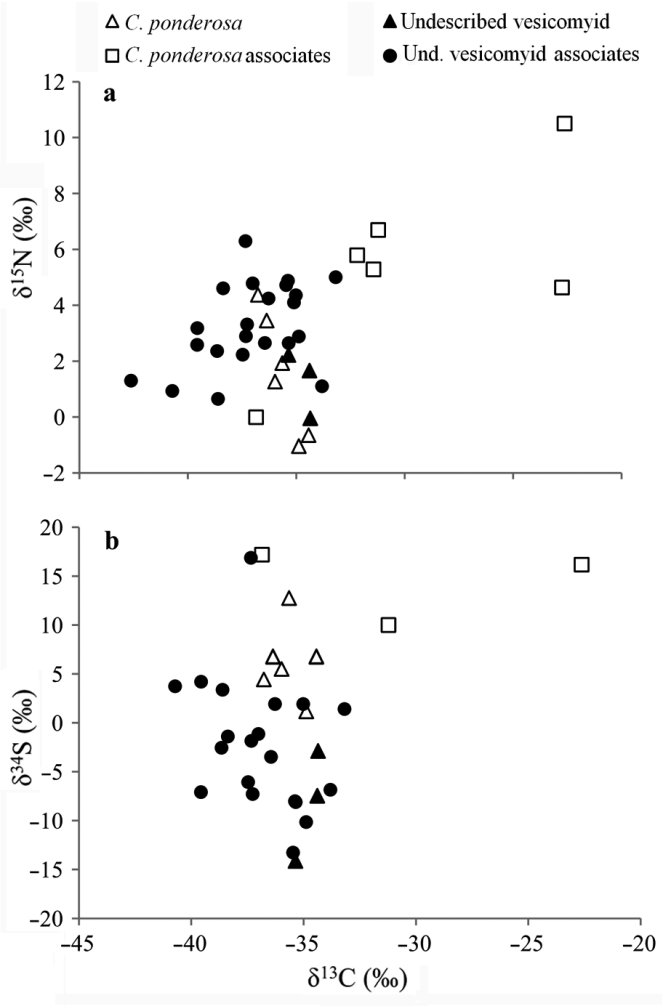

Fig. 4. Tissue (a) $\delta^{15}N$ and (b) $\delta^{34}S$ vs. $\delta^{13}C$ values for the clams *Calyptogena ponderosa* and the undescribed vesicomyid and their associated fauna

lected ($p < 0.001$) (Table S4). This pattern is clearly seen in Fig. 2c, where the points for mean $\delta^{34}S$ values for vestimentiferan collections lie above the 1:1 line and the mussel collections lie below.

$\delta^{15}N$ in mussel and vestimentiferan communities

Compared with $\delta^{13}C$ and $\delta^{34}S$, mean $\delta^{15}N$ values were less variable overall (Table S1, Fig. 2a,b). The average tissue $\delta^{15}N$ values in vestimentiferan collections were both more positive and less variable than in mussel collections (Fig. 2b). For a given vestimentiferan or mussel species, $\delta^{15}N$ values were quite consistent overall, especially within a site (see Becker et al. 2010, 2011). However, average $\delta^{15}N$ values in mussels were approximately 7 to 8‰ more depleted than those of vestimentiferans (Fig. 2b), even within the same study sites. Similarly, macrofauna associated with mussels were significantly more depleted in ^{15}N than vestimentiferan associates (Table S2). Regression analysis revealed a significant linear relationship between the mean $\delta^{15}N$ values of mussels and their associates ($p = 0.001$, $R^2 = 0.49$) but not between vestimentiferans and their associates ($p = 0.53$, $R^2 = 0.05$) (Table 1). GLMs revealed symbiotic fauna type and collection to be significant factors in determining $\delta^{15}N$ values when vestimentiferan and mussel collections were pooled, and collection was also significant for each animal group when considered separately (Table S3).

Paired *t*-tests between mean associated and symbiotic fauna $\delta^{15}N$ by collection revealed that, in both vestimentiferan and mussel collections, the associated macrofauna had significantly greater mean $\delta^{15}N$ values than the symbiotic fauna with which they were collected ($p < 0.001$ for both) (Fig. 2b, Table 1).

Tissue stable isotopes in vesicomyid clam communities

One of the 4 clam collections was obtained with the scoop net and contained a variety of associated animals, while the other 3 collections were obtained by picking up individual clams with the manipulator; therefore, the latter only contained associated fauna that were attached to the clam shells. The undescribed vesicomyid clam was collected only from AC601 (the scoop collection) and *Calyptogena ponderosa* from the other 3 sites (manipulator collections).

Clams ranged in $\delta^{13}C$ from −36.8 to −34.4‰, $\delta^{15}N$ from −1.0 to 4.4‰, and $\delta^{34}S$ from −14.1 to 12.8‰ (Table 2, Fig. 4). The undescribed vesicomyid from AC601 did not differ significantly in $\delta^{13}C$ and $\delta^{15}N$ from the *Calyptogena ponderosa* in other collections ($p = 0.11$ and 0.69 for $\delta^{13}C$ and $\delta^{15}N$, respectively), but $\delta^{34}S$ in the undescribed vesicomyid from AC601 was significantly more depleted in ^{34}S than *C. ponderosa* from other sites ($p < 0.001$). The fauna associated with the undescribed vesicomyid collection had similarly more depleted $\delta^{34}S$ values than the fauna associated with the *C. ponderosa* collections (of which there were only 3 individuals, a polychaete *Glycera* sp., a commensal nautilliniellid, and an anemone (Actinaria), with enough material for $\delta^{34}S$ analyses) (Fig. 4b). Two-sample *t*-tests showed significant differences in $\delta^{13}C$ and $\delta^{34}S$ between clams and their associates ($p = 0.02$ for $\delta^{13}C$, $p = 0.003$ for $\delta^{34}S$) but no significant difference for $\delta^{15}N$ ($p = 0.17$).

DISCUSSION

Tissue $\delta^{13}C$ values implicate biogenic methane as an important nutritional carbon source for lower-slope seep animals

Thermogenic methane in the Gulf of Mexico, which is the dominant form of methane on the upper slope, has $\delta^{13}C$ values ranging from −55 to −30‰ (Roberts & Aharon 1994). This range overlaps substantially with the range of tissue $\delta^{13}C$ values in animals whose nutrition is derived primarily from sulfur-based fixation of seawater dissolved inorganic carbon (DIC). For instance, chemosymbiotic clams from vents and seeps the world over, whose symbionts are known to fix seawater DIC via sulfide oxidation, have tissue $\delta^{13}C$ values between −40 and −30‰ (Fisher 1990, Kennicutt et al. 1992). This overlap has led to ambiguity in differentiating the carbon sources for some seep animals on the upper slope, since both methane and sulfur-based chemoautotrophy can be prevalent in the seep environment.

Biogenic methane in the Gulf of Mexico, on the other hand, has $\delta^{13}C \approx -80$ to −60‰ (Bernard et al. 1977, Roberts & Aharon 1994), making it more isotopically distinct from DIC fixed by sulfur oxidation. Recent studies have shown that biogenic methane is widespread on the lower slope, as indicated by highly negative and spatially variable $\delta^{13}C$ values in porewater methane and authigenic carbonates, and this spatial variability is attributed to differences in the relative proportion of thermogenic and biogenic methane in the local methane pool (Joye et al. 2010, Roberts et al. 2010). Thus, the lower slope offers a system to unambiguously detect the contribution of methane-derived carbon to the nutrition of seep animals. Indeed, very negative and spatially variable tissue $\delta^{13}C$ values have already been shown for mussels and vestimentiferan tubeworms on the lower slope, implicating methane-derived carbon as an important carbon source for both symbioses, despite the differences in their symbionts' carbon fixation pathways (Becker et al. 2010, 2011).

In the present study, we looked for a significant contribution of methane to the macrofaunal community associated with mussels and vestimentiferans. Approximately one quarter of all fauna associated with mussels and vestimentiferans had $\delta^{13}C$ values <−55‰. Most of the associated macrofauna with tissue $\delta^{13}C$ values lower than −60‰ were associated with mussels, which was expected because of the greater abundance of methane above the sediment surface in mussel habitats (Nix et al. 1995). More unexpectedly, approximately half of the fauna associated with vestimentiferans had $\delta^{13}C$ values of −55 to −45‰, indicating incorporation of methane-derived carbon.

It is well-established that vestimentiferans mine sulfide from the sediment using the buried posterior portion of their body, or 'root' (Freytag et al. 2001), allowing them to persist well after flux of seeping fluids to the sediment surface has slowed. Indeed, much lower levels of sulfide have been measured in the first 10 cm of sediment surrounding older vestimentiferan aggregations (Cordes et al. 2005a, 2006) than young aggregations, and much higher levels have been measured in the deeper layers of sediment surrounding the vestimentiferan roots than in the surface layer (Julian et al. 1999, Cordes et al. 2005b).

Recent evidence suggests that vestimentiferans also use their roots to take up methane-derived DIC from the sediment (Dattagupta et al. 2006, Becker et al. 2011). Since most of the vestimentiferan aggregations collected in the present study were comprised of larger (older) individuals, and concentrations of methane and sulfide are often positively correlated (Nix et al. 1995), it was reasonable to hypothesize methane-derived DIC may be available to vestimentiferan roots but largely unavailable to the free-living microbes at the sediment-seawater interface. Because these microbes form the base of the associated macrofaunal food web, the highly negative $\delta^{13}C$ values in the associated fauna indicate that methane-derived DIC was available in sufficient quantities at the sediment-seawater interface to produce a detectable signal in the macrofauna. In both mussel and vestimentiferan communities, the congruent pattern of spatial variability in tissue $\delta^{13}C$ of associates and the symbiotic species in the same local environment provides further evidence for the importance of methane to both trophic groups, despite the large differences in their modes of nutritional carbon acquisition.

$\delta^{34}S$ in mussel and vestimentiferan communities: implications for fractionation during sulfate reduction

Sulfate and sulfide are the primary forms of inorganic sulfur available at seeps. In Gulf of Mexico seep sediments, microbial hydrocarbon oxidation coupled with sulfate reduction produces the majority of available sulfide (Boetius et al. 2000, Joye et

al. 2004, Orcutt et al. 2005). The process of sulfate reduction discriminates against ^{34}S, producing isotopically depleted sulfide where sulfate is not limited (Chambers & Trudinger 1979). Sulfate reduction rate and sulfate limitation can affect the amount of isotopic fractionation, where higher rates and limited sulfate lead to less isotopic discrimination (Aharon & Fu 2000, Joye et al. 2009). We found extreme variability in tissue $\delta^{34}S$ within collections (e.g. associated fauna $\delta^{34}S$ = −17 to +12‰ in a single vestimentiferan collection), reflecting the potential for small-scale variability. Nonetheless, as with $\delta^{13}C$, we observed significant spatial variation in tissue $\delta^{34}S$ values and a significant linear relationship between the tissue $\delta^{34}S$ of associates and symbiotic fauna from the same locations. Thus, despite the small-scale variability, the $\delta^{34}S$ of the local inorganic sulfur pool influences the tissue $\delta^{34}S$ values of the entire local community.

Vestimentiferans, clams, and their associates had more depleted and more variable $\delta^{34}S$ values than mussels and their associates. This difference is consistent with data that show less fractionation, and therefore isotopically enriched sulfide, where methane is abundant (Aharon & Fu 2003) as is typical in mussel habitats (Nix et al. 1995, Bergquist et al. 2005). Additionally, the vestimentiferans and clams themselves can alter sediment sulfur chemistry. Vestimentiferans and clams take up sulfide from the sediment, vestimentiferans release sulfate directly into the sediment through their roots (Cordes et al. 2005b, Dattagupta et al. 2006), and clams burrow through the sediment and increase mixing at the sediment-seawater interface (Treude et al. 2003).

Though more enriched than vestimentiferans, the average $\delta^{34}S$ values in mussels and their associates are still depleted relative to non-seep animals whose nutritional sulfur is derived primarily from seawater sulfate (Rees 1978), indicating that sulfide-derived organic sulfur is also important in mussel habitats. In one collection of *Bathymodiolus brooksi* from AC645, the mussels and their associates were highly depleted in $\delta^{34}S$ relative to other mussel collections and also had the most enriched $\delta^{13}C$ values relative to all other mussel collections in this study. *B. brooksi* hosts both sulfur- and methane-oxidizing symbionts, and the very low $\delta^{34}S$ values in the mussels and associated fauna could indicate isotopically depleted sulfide in the environment or a higher nutritional contribution from sulfur oxidizing bacteria—both symbiotic and free-living—relative to other mussel collections.

Factors affecting tissue $\delta^{15}N$ in mussel and vestimentiferan communities

$\delta^{15}N$ values for most animals in this study were much lower than photosynthesis derived particulate organic nitrogen (PON $\delta^{15}N$ = 2.5 to 23.3‰ at depth >200 m; Saino & Hattori 1987), indicating use of a local nitrogen source. The mechanisms by which local inorganic nitrogen becomes incorporated into the seep food web are not well-known; however, assimilation of ammonium, nitrate, and free amino acids has been documented in the mussel *Bathymodiolus childressi* (Lee et al. 1992, Lee & Childress 1994, 1996), and some free-living microbes likely do this as well. The isotope compositions of ammonium and nitrate in seep sediments have not been measured, but it has been hypothesized that assimilation of ammonium could lead to high isotopic fractionation (Hoch et al. 1994, Lee & Childress 1996). A previous study demonstrated nitrogenase activity in coastal marine sediments with moderate ammonium concentrations (Bertics et al. 2010), but fixation of local molecular nitrogen (N_2) has yet to be demonstrated as a significant nitrogen source in seep sediments.

The significantly more depleted $\delta^{15}N$ values of mussels and their associated fauna compared with vestimentiferans and their associates suggests a difference in the inorganic nitrogen pools between their respective habitats or the presence of more higher-order consumers in vestimentiferan food webs (Table S2). The significant linear relationship between the $\delta^{15}N$ of mussels and their associates further supports an environmental driver for the spatial variation in $\delta^{15}N$ values in these communities. The lack of a significant linear relationship between the $\delta^{15}N$ values of vestimentiferans and their associates could be due to the low among-collection variability in average $\delta^{15}N$ values compared with mussel collections or a difference between free-living microbes and vestimentiferan symbionts in the acquisition or assimilation of inorganic nitrogen.

Factors affecting tissue stable isotope values in vesicomyid clam communities

The $\delta^{13}C$ and $\delta^{15}N$ values of *Calyptogena ponderosa* and the undescribed vesicomyid in this study are consistent with the narrow range of tissue $\delta^{13}C$ and $\delta^{15}N$ documented in vent and seep vesicomyids clams the world over ($\delta^{13}C$ = −40 to −30‰ and $\delta^{15}N$ = −3 to 8‰; Kennicutt et al. 1992, Fisher 1990). The

lack of spatial variability in clam tissue $\delta^{13}C$ values is consistent with their symbionts fixing oceanic (not porewater) DIC via sulfur oxidation. The fixation pathway likely proceeds using form II RuBisCO, the same form that has been found in all vesicomyid symbionts that have thus far been genetically analyzed (Newton et al. 2008).

The $\delta^{34}S$ values, by contrast, were significantly more depleted in the undescribed vesicomyid from AC601 than from *Calyptogena ponderosa* from the other 3 sites. The $\delta^{34}S$ fauna associated with the clams show a similar pattern, with significantly lower tissue $\delta^{34}S$ values from the AC601 undescribed vesicomyid collection than the 3 *C. ponderosa* collections (although only 3 animals were collected with *C. ponderosa*). The matching difference in the tissue $\delta^{34}S$ of associated macrofauna and clams suggests that the $\delta^{34}S$ composition of the endmember sulfur source (i.e. porewater sulfide), and not physiological differences between clam species, drives the difference in sulfur isotopes of the 2 clam species and their associates.

CONCLUSIONS

Together, the findings in this study highlight that (1) the $\delta^{13}C$ composition of the local methane pool influences the entire local food web, even in vestimentiferan communities; (2) where the $\delta^{13}C$ signature of the methane differs greatly from that of chemoautotrophically fixed seawater DIC, tissue $\delta^{13}C$ values can be a good indicator of the endmember inorganic carbon source; but (3) $\delta^{13}C$ values are *not* a reliable indicator of the relative importance of methanotrophy vs. chemoautotrophy. This is best illustrated by the vestimentiferans, which have only sulfur-oxidizing symbionts, and yet their tissue $\delta^{13}C$ values are often similar to carbon fixed by methanotrophy. The local inorganic nitrogen pool clearly influences the $\delta^{15}N$ values of the entire local community in mussel habitats, but the lack of stable isotope data for inorganic nitrogen sources in seep sediments or knowledge of the specific chemical species used by vestimentiferans hinders the use of $\delta^{15}N$ to discern trophic position. Though tissue $\delta^{34}S$ values can point toward a chemoautotrophic food source, their extreme variability in seep animals reflects a complex sulfur cycle influenced not only by microbial and geochemical processes, but also the dominant seep megafauna (e.g. vestimentiferans and vesicomyids). A better understanding of the underlying causes of the spatial variability in tissue $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values is needed before they can be reliably used in mixing models or construction of theoretical food webs.

Data availability. The original stable isotope data presented in this manuscript are available on the U.S. Geological Survey Ocean Biogeographic Information System found at www.usgs.gov/obis-usa/

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LITERATURE CITED

Aharon P, Fu B (2000) Microbial sulfate reduction rates and sulfur and oxygen isotope fractionations at oil and gas seeps in deepwater Gulf of Mexico. Geochim Cosmochim Acta 64:233–246

Aharon P, Fu B (2003) Sulfur and oxygen isotopes of coeval sulfate-sulfide in pore fluids of cold seep sediments with sharp redox gradients. Chem Geol 195:201–218

Becker EL, Lee RW, Macko SA, Faure BM, Fisher CR (2010) Stable carbon and nitrogen isotope compositions of hydrocarbon seep bivalves on the Gulf of Mexico lower continental slope. Deep-Sea Res II 57:1957–1964

Becker EL, Macko SA, Lee RW, Fisher CR (2011) Insights into seep vestimentiferan physiological ecology from tissue stable isotope analysis. Naturwissenschaften 98: 169–174

Bergquist DC, Ward T, Cordes E, McNelis T and others (2003) Community structure of vestimentiferan-generated habitat islands from Gulf of Mexico cold seeps. J Exp Mar Biol Ecol 289:197–222

Bergquist DC, Fleckenstein C, Knisel J, Begley B, MacDonald IR, Fisher CR (2005) Variations in seep mussel bed communities along physical and chemical environmental gradients. Mar Ecol Prog Ser 293:99–108

Bernard B, Brooks JM, Sackett WM 1977. A geochemical model for characterization of gas sources in marine sediments. Offshore Technology Conference, Houston, TX, p 435–438

Bertics VJ, Sohm JA, Treude T, Chow CET, Capone DG, Fuhrman JA, Ziebis W (2010) Burrowing deeper into

benthic nitrogen cycling: the impact of bioturbation on nitrogen fixation coupled to sulfate reduction. Mar Ecol Prog Ser 409:1–15

Boetius A, Ravenschlag K, Schubert CJ, Rickert D and others (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407: 623–626

Carlier A, Ritt B, Rodrigues CF, Sarrazin J, Olu K, Grall J, Clavier J (2010) Heterogeneous energetic pathways and carbon sources on deep eastern Mediterranean cold seep communities. Mar Biol 157:2545–2565

Cavanaugh CM, Levering PR, Maki JS, Mitchell R, Lidstrom ME (1987) Symbiosis of methylotrophic bacteria and deep-sea mussels. Nature 325:346–348

Chambers LA, Trudinger PA (1979) Microbiological fractionation of stable sulfur isotopes: a review and critique. Geomicrobiol J 1:249–293

Cordes EE, Hourdez S, Predmore BL, Redding ML, Fisher CR (2005a) Succession of hydrocarbon seep communities associated with the long-lived foundation species *Lamellibrachia luymesi.* Mar Ecol Prog Ser 305:17–29

Cordes EE, Arthur MA, Shea K, Arvidson RS, Fisher CR (2005b) Modeling the mutualistic interactions between tubeworms and microbial consortia. PLoS Biol 3:e77

Cordes EE, Bergquist DC, Predmore BL, Jones C, Deines P, Telesnicki G, Fisher CR (2006) Alternate unstable states: convergent paths of succession in hydrocarbon-seep tubeworm-associated communities. J Exp Mar Biol Ecol 339:159–176

Cordes EE, Becker EL, Hourdez S, Fisher CR (2010) Influence of foundation species, depth, and location on biodiversity and structure of cold seep macrofaunal communities on the lower slope of the Gulf of Mexico. Deep-Sea Res II 57:1870–1881

Cordes EE, Becker EL, Fisher CR (2010b) Temporal shift in nutrient input to cold-seep webs revealed by stable-isotope signatures of associated communities. Limnol Oceanogr 55:2537–2548

Dattagupta S, Miles LL, Barnabei MS, Fisher CR (2006) The hydrocarbon seep tubeworm *Lamellibrachia luymesi* primarily eliminates sulfate and hydrogen ions across its roots to conserve energy and ensure sulfide supply. J Exp Biol 209:3795–3805

Duperron S, Sibuet M, MacGregor BJ, Kuypers MMM, Fisher CR, Dubilier N (2007) Diversity, relative abundance and metabolic potential of bacterial endosymbionts in 3 *Bathymodiolus* mussel species from cold seeps in the Gulf of Mexico. Environ Microbiol 9:1423–1438

Fisher CR (1990) Chemoautotrophic and methanotrophic symbioses in marine invertabrates. Aquat Sci 2: 399–436

Fisher CR, Brooks JM, Vodenichar JS, Zande JM, Childress JJ, Burke RA (1993) The co-ocurrance of methanotrophic and chemoautotrophic sulfur-oxidizing bacterial symbionts in a deep-sea mussel. Mar Ecol 14:277–289

Freytag JK, Girguis PR, Bergquist DC, Andras JP, Childress JJ, Fisher CR (2001) A paradox resolved: sulfide acquisition by roots of seep tubeworms sustains net chemoautotrophy. Proc Natl Acad Sci USA 98:13408–13413

Fry B, Gest H, Hayes JM (1983) Sulfur isotopic compositions of deep-sea hydrothermal vent animals. Nature 306:51–52

Gearing JN, Gearing PJ, Rudnick DT, Requejo AG, Hutchins MJ (1984) Isotopic variability of organic carbon in a phytoplankton-based, temperate estuary. Geochim Cosmochim Acta 48:1089–1098

Hoch MP, Fogel ML, Kirchman DL (1994) Isotope fractionation during ammonium uptake by marine microbial assemblages. Geomicrobiol J 12:113–127

Holm S (1979) A simple sequentially rejective multiple test procedure. Scand J Stat 6:65–70

House CH, Orphan VJ, Turk KA, Thomas B, Pernthaler A, Vrentas JM, Joye SB (2009) Extensive carbon isotopic heterogeneity among methane seep microbiota. Environ Microbiol 11:2207–2215

Joye SB, Boetius A, Orcutt BN, Montoya JP, Schulz HN, Erickson MJ, Lugo SK (2004) The anaerobic oxidation of methane and sulfate reduction in sediments from Gulf of Mexico cold seeps. Chem Geol 205:219–238

Joye SB, Samarkin VA, Orcutt BN, MacDonald IR and others (2009) Metabolic variability in seafloor brines revealed by carbon and sulphur dynamics. Nat Geosci 2: 349–354

Joye SB, Bowles MW, Samarkin VA, Hunter KS, Niemann H (2010) Biogeochemical signatures and microbial activity of different cold seep habitats along the Gulf of Mexico lower slope. Deep-Sea Res II 57:1990–2001

Julian D, Gaill F, Wood E, Arp AJ, Fisher CR (1999) Roots as a site of hydrogen sulfide uptake in the hydrocarbon seep vestimentiferan *Lamellibrachia* sp. J Exp Biol 202: 2245–2257

Kennicutt MC, Burke RA, MacDonald IR, Brooks JM, Denoux GJ, Macko SA (1992) Stable isotope partitioning in seep and vent organisms: chemical and ecological significance. Chem Geol 101:293–310

Lee RW, Childress JJ (1994) Assimilation of inorganic nitrogen by marine invertebrates and their chemoautotrophic and methanotrophic symbionts. Appl Environ Microbiol 60:1852–1858

Lee RW, Childress JJ (1996) Inorganic N assimilation and ammonium pools in a deep-sea mussel containing methanotrophic endosymbionts. Biol Bull (Woods Hole) 190: 373–384

Lee RW, Thuesen EV, Childress JJ, Fisher CR (1992) Ammonium and free amino acid uptake by a deep-sea mussel (*Bathymodiolus* sp., undescribed) containing methanotrophic bacterial symbionts. Mar Biol 113:99–106

Levin LA (2005) Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. In: Gibson RN, Atkinson RJA, Gordon JDM (eds) Oceanography and marine biology—an annual review, Vol 43. CRC Press, Boca Raton, FL, p 1–46

Levin LA, Mendoza GF (2007) Community structure and nutrition of deep methane-seep macrobenthos from the North Pacific (Aleutian) Margin and the Gulf of Mexico (Florida Escarpment). Mar Ecol 28:131–151

Levin LA, Michener RH (2002) Isotopic evidence for chemosynthesis-based nutrition of macrobenthos: the lightness of being at pacific methane seeps. Limnol Oceanogr 47: 1336–1345

MacAvoy SE, Fisher CR, Carney RS, Macko SA (2005) Nutritional associations among fauna at hydrocarbon seep communities in the Gulf of Mexico. Mar Ecol Prog Ser 292:51–60

Miglietta MP, Hourdez S, Cowart DA, Schaeffer SW, Fisher CR (2010) Species boundaries of Gulf of Mexico vestimentiferans (Polychaeta, Siboglinidae) inferred from mitochondrial genes. Deep-Sea Res II 57:1916–1925

Newton ILG, Girguis PR, Cavanaugh CM (2008) Comparative genomics of vesicomyid clam (Bivalvia: Mollusca) chemosynthetic symbionts. BMC Genomics 9:585

Nix ER, Fisher CR, Vodenichar J, Scott KM (1995) Physiological ecology of a mussel with methanotrophic endosymbionts at three hydrocarbon seep sites in the Gulf of Mexico. Mar Biol 122:605–617

Orcutt B, Boetius A, Elvert M, Samarkin V, Joye SB (2005) Molecular biogeochemistry of sulfate reduction, methanogenesis and the anaerobic oxidation of methane at Gulf of Mexico cold seeps. Geochim Cosmochim Acta 69: 4267–4281

Orcutt BN, Joye SB, Kleindienst S, Knittel K and others (2010) Impact of natural oil and higher hydrocarbons on microbial diversity, distribution, and activity in Gulf of Mexico cold-seep sediments. Deep-Sea Res II 57:2008–2021

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320

Rees CE (1978) Sulphur isotope measurements using SO_2 and SF_6. Geochim Cosmochim Acta 42:383–389

Roberts H, Aharon P (1994) Hydrocarbon-derived carbonate buildups of the northern Gulf of Mexico continental slope: a review of submersible investigations. Geo-Mar Lett 14:135–148

Roberts HH, Feng D, Joye S (2010) Cold seep carbonates of the middle and lower Continental Slope, Northern Gulf of Mexico. Deep-Sea Res II 57:2040–2054

Saino T, Hattori A (1987) Geographical variation of the water column distribution of suspended particulate organic nitrogen and its ^{15}N natural abundance in the Pacific and its marginal seas. Deep-Sea Res 34: 807–827

Treude T, Boetius A, Knittel K, Wallmann K, Jørgensen BB (2003) Anaerobic oxidation of methane above gas hydrates at Hydrate Ridge, NE Pacific Ocean. Mar Ecol Prog Ser 264:1–14

Urcuyo IA, Massoth GJ, Julian D, Fisher CR (2003) Habitat, growth and physiological ecology of a basaltic community of *Ridgeia piscesae* from the Juan de Fuca Ridge. Deep-Sea Res I 50:763–780

Van Dover CL (2002) Community structure of mussel beds at deep-sea hydrothermal vents. Mar Ecol Prog Ser 230: 137–158

Zar JH (2010) Biostatistical analysis, 5th edn. Pearson Prentice-Hall, Upper Saddle River, NJ

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